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Procedia Engineering 18 (2011) 72 – 77

Procedia Engineering

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The Second SREE Conference on Chemical Engineering

Absorption and oxidation of arsenite by *Pteris vittata* roots and its kinetics

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Abstract

In this paper, hydroponic experiment was employed to study the absorption and oxidation of arsenite by *Pteris vittata* roots segment. The results showed that arsenite could be oxidized to arsenate by *Pteris vittata*s roots in a short time. The maximum percentage of arsenite oxidized to arsenate was obtained from different concentration of arsenite culture solution within 8h. The absorption ability of arsenite by *Petris vittata* roots cultured in low concentration arsenite solution was higher than that in high concentration arsenite solution. According to the modified *Michaelis-Menten* equation, V_{max} and K_m were calculated by using *Line-weaver-Burk* illustration method from the 10 $mg \cdot L^{-1}$ arsenite experimental data, which were (0.183±0.006) $mg \cdot h^{-1} \cdot g^{-1}$ and (2.590±0.080) $mg \cdot L^{-1}$ respectively.

Keywords: *Pteris vittata roots*, arsenite, absorption characteristics, absorption rate constant;

1. Introduction

Arsenic (As) is ubiquitous in the environment and its presence in soils is due to both geogenic and anthropogenic sources. Arsenic is also a carcinogenic and mutagenic element, therefore it can cause acute or chronic poisoning when body uptake a certain dose of arsenic. Arsenic is ranked as the No.5 soil pollutant caused by irrigation in China [1], and it also has been classified as priority pollutant nationally and internationally Remediation of arsenic-contaminated water has become a major environmental issue.

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doi:10.1016/j.proeng.2011.11.012

Phytoextraction, a plant based technology for the removal of toxic contaminants from soil and water is an attractive approach [2, 3]. Ma et al. [4] reported the first known arsenic hyperaccumulator *Pteris vittata* (Chinese brake fern), which could accumulate large amounts of arsenic (up to 2.3% dry wt.) in its aboveground biomass. Recently years, the community researchers have paid much attention to the characteristics of arsenic hyperaccuulation by *Pteris vittata*. Pentavalent arsenic is absorbed by non-arsenic-resistant plants through the phosphate transport corridor, while the absorption mechanism of trivalent arsenic is still uncertain .The same findings are also reflected in the absorption of arsenic by *Pteris vittata* [5]. Kertulis et al [6] have researched the arsenic concentration in the xylem sap of *Pteris vittata*, and obtained that the arsenate absorption rate by *Pteris vittata* was faster than that of arsenite. Poynton et al [7] found, during the hydroponic experiment, the arsenite flux entering to the *Pteris vittata* roots was significantly larger than that of non-arsenic hyperaccumulator *Nephrolepis exaltata*. In addition, Wang et al [8] found that in hydroponic condition, arsenite absorption rate by *Pteris vittata* was very slow and was probably one tenth of the arsenate absorption rate. Many researchers have analyzed the absorption mechanism of arsenic by *Pteris vittata*, concluding that the capacity of hyperaccumulation is related to the roots absorptive capacity and absorptive rate [9～14]. *Pteris vittata* has a good capacity of absorbing arsenic. Hence it is considered an extremely promising direction of arsenic phytoremediation.

There is little information about the specific characteristics of *Pteris vittata* absorbing arsenite in water. The purpose of this study means to provide theoretical support for the characteristics of arsenic absorption by *Pteris vittata* in water solution, and to calculate the kinetic parameters of absorption rate.

2. Materials and Methods

2.1. Pretreatment

Pteris vittata were collected from *Guangzhou Higher Education Mega Center*, the height are about 30 ~ 35cm, with roughly same biomass and bipinnatas. After removed adhered soils and dusts, the *Pteris vittata* were cultured in plastic pots filled with tap water for three days, then were transferred to plastic pots filled with the nutrient solution which contained (mmol·L^{-1}): $MgSO_4$ 0.2，$K2HPO_4$ 2，NH_4NO_3 2，KNO_3 2，NaCl 2，$CuSO_4 \cdot 5H_2O$ 16×10^{-5}，$ZnSO_4 \cdot 5H_2O$ 4×10^{-4} and were allowed to grow 30 days .The pH of the nutrient solution was controlled at 7.86. The containers for hydroponic plants were 70cm × 20cm × 20cm plastic tanks. The growth room was climate-controlled with a temperature range 15 ~ 20℃ and relative humidity ~55%.The illumination, of which intensity was 5000 ~ 10000Lx, was supplied to illuminate the *Pteris vittata* for ten hours every day. After pre-cultured, *Pteris vittata* roosts were cleaned by deionized water, then were transferred into 100mL deionized water for 24 hours. All reagents used were of analytical grade

2.2. Experimental design

The experiment steps were as follows: Selected 12 *Pteris vittata* which had little difference in height, age, morphology index in plastic pots for hydroponic experiment, added 0mg·L^{-1}，10mg·L^{-1}，20mg·L^{-1}，30mg·L^{-1}，40mg·L^{-1}，50mg·L^{-1}，80mg·L^{-1}，100mg·L^{-1}，150mg·L^{-1}，200mg·L^{-1}，500 mg·L^{-1} and 1000mg·L^{-1} sodium arsenite($NaAsO_2$) culture solution respectively, then determined the arsenite concentration after 0h,1h,2h,4h,6h,8h,10h and 24h respectively. At the same time, the *Pteris vittata* roots should be washed with deionized water to release the arsenic adsorbed by *Pteris vittata* before taking test solution samples. Deinonized water was added into the plastic tanks every one hour to supplement water loss due to absorption, transpiration, etc. Five replicates were used in each treatment for the experiment.

The concentration of arsenite in culture solution was determined by amino acid diethyl sulfide silver colorimetry. Analyte solution was added concentrated hydrochloric acid, and then was extracted in toluene solution. The molar quantities of the arsenite in the organic phase were determined by iodine titration. The arsenate in the water phase was reduced to arsenite by $TiCl_3$ and determined by iodine titration. The *Pteris vittata* roots were separated, dewatered, dried, crushed to obtain homogeneous samples which were digested by microwave digestion method and were analyzed its arsenite concentration by plasma emission spectrometry (IPC) method.

2.3. Statistical analysis

To make sure the validity of the data, all results were expressed by an average of five parallel experiments. Experiments effects were determined by analyzing the variance, and significant effects on the single factor were also assessed. All statistical procedures were carried out by using OriginPro7.5 software.

3. Results and Discussion

3.1. The oxidation of arsenite by Pteris vittatas roots in culture solution

Arsenite was transferred to *Pteris vittata* by two ways, i.e.(1)being absorb directly by *Pteris vittata*s;(2) being oxidize to arsenate and then to be absorbed by *Pteris vittata*s. Arsenite can be oxidized to arsenate in a short time by *Pteris vittata*s roots [15]. The concentration of arsenate in culture solution was determined after 0h, 1h, 2h, 4h, 6h, 8h, 10h and 24h respectively.

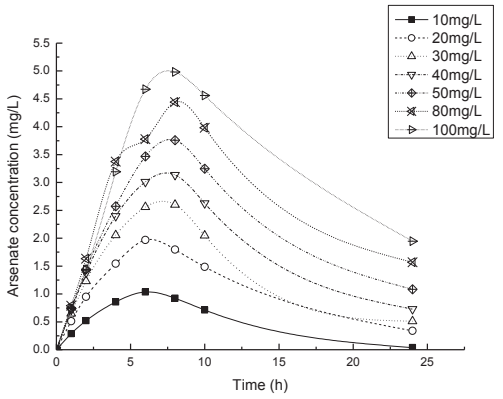

Fig1 Arsenate concentration in solution when supplied with 10^{-1}00 mg·L^{-1} arsenite

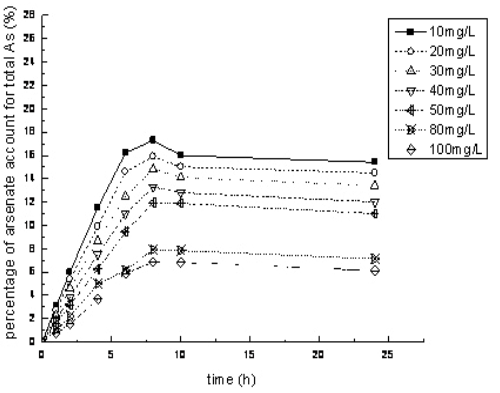

Fig2 Percentage of Arsenate for taking up of total arsenic in solution

When the initial concentration of arsenite was 10mg·L^{-1} and 20mg·L^{-1} respectively, the concentration of arsenate oxidized from arsenite in solution can achieve its maximum within 6h, which were 1.04 mg·L^{-1} and 1.97 mg·L^{-1} respectively. While it was taken 8h for other culture solution to obtain maximum arsenate, which was 2.60 mg·L^{-1} in 30 mg·L^{-1} arsenite culture solution and was 4.98 mg·L^{-1} in 100 mg·L^{-1} arsenite culture solution（Fig1）. After 8h, the concentration of arsenate began to decrease. It can be explained by two reasons: (1) the rate of arsenite oxidation was slowing with the decreasing of arsenite in

culture solution; (2) arsenate was absorbed by *Pteris vittata*s roots [15]. The phenomenon was also revealed in Fig2, the percentage of arsenite oxidized to arsenate in solution was decreasing after 8h.The percentage of arsenate for taking up of the total arsenic in solution was increasing linearly with time in the period of 0～6h after treatment, excepting 80 $mg \cdot L^{-1}$ arsenite solution. It was illustrated from Fig2 that the percentage of arsenate for taking up of the total arsenic in solution was decreasing with the increase of initial concentration of arenite in culture solution. The maximum arsenate percentage was 17.3% in 10 $mg \cdot L^{-1}$ arsenite culture solution after 8h while the minimum one was 6.86% in 100 $mg \cdot L^{-1}$ after 8h.

3.2. Absorption percentage of arcenite by Petris vittata roots

Table 1 showed the amount of arsenite in *Pteris vittata* roots after 24h. The percentage of arsenite accumulated in *Pteris vittata* roots increased with the decrease of initial concentration of arsenite in culture solution. With the increasing amount of the arsenite in *Pteris vittata* roots, the arsenite were transferred to other parts of *Pteris vittata*. It indicated that the arsenite absorption ability of *Pteris vittata* roots in low concentration arsenite culture solution was stronger than that in high concentration arsenite culture solution, thus the enrichment efficiency of arsenite by roots decreased with the increase of the initial concentration of arsenite in culture solution.

Table 1.The content of arsenite which absorbed by roots segment of *Pteris vittata* when initial arsenite was different

The concentration of arsenite in hydroponics/($mg \cdot L^{-1}$)	10	20	30	40	50	60	80	100	150	200	500	1000
The amount of arsenite in *Pteris vittata* roots/($mg \cdot Kg^{-1}$)	110	115	153	158	165	173	185	116	145	170	250	375
The bioconcentration factor of roots	11.00	5.75	5.10	3.95	3.30	2.88	2.30	1.16	0.97	0.85	0.50	0.375

The ANOVA of repeated test is showed in table 2. The absorption differences of arsenite among the different arsenite culture solution reached significant level of 1%. It indicated that the change of the concentration of arsenite in culture solution would cause significant influence in the absorption of arsenite by *Pteris vittata*.

Table 2.The variance analysis of the content of arsenite which absorbed by roots segment of *Pteris vittata* when initial arsenite was different

Different sources	Sum of squares	Degrees of freedom	Variance	F	P
interior-group	294071.25	11	26733.75	663.506	$P<0.01$
inter-group	1934	48	40.2917		
summation	296005.25	59			

3.3. The absorption rate constant of arsenite by Petris vittata

The absorption rate of arsenite by *Pteris vittata* meant the ratio of the amount of absorption arsenite by *Pteris vittata* in a unit time to the fresh weight of *Pteris vittata* roots, its unit is $mg \cdot h^{-1} \cdot g^{-1}$. The absorption rate were determined and analysed every 2 hours. The results showed that: the absorption rate of different time kept more consistent in low concentration asrenite culture solution (<50 $mg \cdot L^{-1}$), while they were

more different when in high concentration (>50 mg·L^{-1}) (Fig3).The reason was probably that arsenite have been oxidized to arsenate, it need to be further study.

The Fig3 was plotted according to the average absorption rate of the former 4h of different concentration arsenite culture solution. The minimal absorption rate was about 0.13mg·h^{-1}·g^{-1}, appearing in 10mg·L^{-1} arsenite culture solution .While the maximum was about 0.45mg·h^{-1}·g^{-1} in 50mg·L^{-1} arsenite culture solution. The phenomenon was due to the difference of arsenite concentration in culture solution, the absorption rate increased with the increase of arsenite concentration (Fig3).

Releasing arsenite and absorbing arsenite by *Pteris vittata* happened at same time. When the concentration of arsenite decreased to a very low level, the absorption rate achieved equilibrium with the release rate [7]. Hence it could achieved equilibrium within 24h when the initial concentration of arsenite was 10 mg·L^{-1}. Fig4 showed that the arsenite concentration changed with time when the initial concentration of arsenite was 10mg·L^{-1}. 24h after treatment, the absorption rate and release rate achieved equilibrium in 10mg·L^{-1} arsenite culture solution, the concentration of arsenite was 0.21mg·L^{-1} at this time.

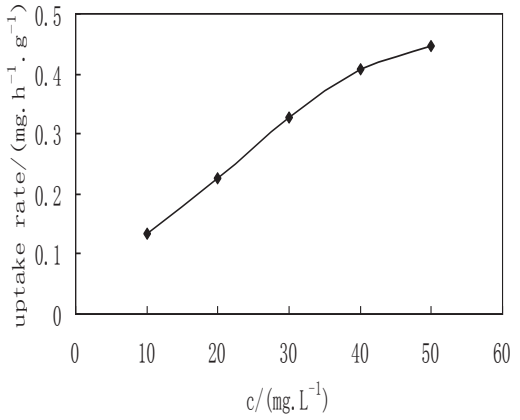

Fig. 3 The average absorption rates of arsenite by *Pteris vittata* in different initial solution

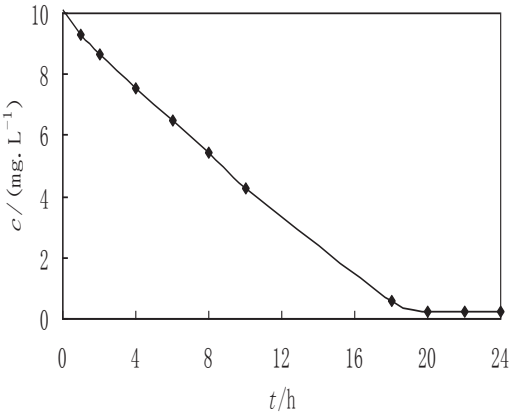

Fig. 4 Changes of the arsenite concentration when initial arsenite was 10 mg·L^{-1} within 24 hours

Since *pteris vittata* absorbing arsenite was the process of enzymatic reaction, the uptake kinetic parameters could be calculated by using modified Michaelis-Menten equation [15].The equation was as follow:

$$\frac{1}{v_i} = \frac{K_m}{v_{\max}(C_i - C_{\min})} + \frac{1}{v_{\max}} \qquad (1)$$

$C_{\min}$ was the concentration of arsenite when the absorption rate has achieved equilibrium with release rate, C_i and v_i was the arsenite concentration and arsenite absorption rate by *Pteris vittata* in different time respectively, and $v_{\max}$ could be educed by *Lineweaver-Burk* graph charted with $1/v_i$ versus $1/(C_i - C_m)$, $v_{\max}$ represented the arsenite maximal absorption rate by *Pteris vittata* roots, K_m reflected the affinity of *Pteris vittata* to arsenite . The calculative results were: K_m 2.590 ± 0.080

$mg \cdot L^{-1}$, v_{max}（0.183 ± 0.006） $mg \cdot h^{-1} \cdot g^{-1}$, C_{min} （0.217 ± 0.012） $mg \cdot L^{-1}$, r^2（0.940 ± 0.010）（r^2 is the correlation coefficient of the equation）.

4. Conclusion

17.3% arsenite can oxidize to arsenate by *Pteris vittatas* roots within 8h when the culture solution was treated with 10 $mg \cdot L^{-1}$ arsenite. And over 6% of arsenite have been oxidized to arsenate in 20 $mg \cdot L^{-1}$, 30 $mg \cdot L^{-1}$, 40 $mg \cdot L^{-1}$, 50 $mg \cdot L^{-1}$, 80 $mg \cdot L^{-1}$ and 100 $mg \cdot L^{-1}$ arsenite culture solution within 8h. The absorption ability of arsenite by *Pteris vittata* roots cultured in low concentration arsenite solution was higher than that in high concentration arsenite solution .It was because the roots absorption capacity would been inhibited when the concentration of arsenite in solutuion increased to a level. Finally, according to Michaelis-Menten equation and by using fitting chart to calculate the kineti parameters of enzymatic reaction, the result was obtained as follow: v_{max} (0.183±0.006) $mg \cdot h^{-1} \cdot g^{-1}$, K_m (2.590±0.080) $mg \cdot L^{-1}$

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